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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/799,417	03/12/2004	Paul A. Krieg	20825-0004	6904

7590 12/10/2007

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EXAMINER	
BRISTOL, LYNN ANNE	

ART UNIT	PAPER NUMBER
1643	

MAIL DATE	DELIVERY MODE
12/10/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/799,417	KRIEG, PAUL A.	
	Examiner	Art Unit	
	Lynn Bristol	1643	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *19 March 2007*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-13,15,16,18-26 and 28-30* is/are pending in the application.

4a) Of the above claim(s) *15,16 and 18-20* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-13,21-26 and 28-30* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *9/19/07*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Claims 1-13, 15, 16, 18-26, 28-30 are all the pending claims for this application.

2. Claims 14, 17 and 31 were cancelled and Claims 1, 6, 8, 22, 26, 28 and 30 were amended in the Response of 9/19//07

3. Claims 15, 16 and 18-20 are withdrawn from examination.

4. Claims 1-13, 21-26 and 28-30 are all the pending claims under examination.

5. Applicants amendments to the claims and arguments for enablement have necessitated new grounds for rejection. **This action is FINAL**.

Information Disclosure Statement

6. The non-patent references filed in the IDS of 9/19/07 have been considered and entered. An initialed copy of the 1449 form is attached.

Withdrawal of Objections

Sequence Listing/ New Matter

7. The Preliminary Amendment of 10/7/05 requesting entry of a revised Sequence Listing in order to correct a sequence error in SEQ ID NO: 11 has been considered and entered.

Applicant's statement on pp. 9-10 of the Response of 9/19/07 that the revision to the Sequence Listing for SEQ ID NO:11 to properly identify the sequence as a nucleic acid rather than an amino acid

Specification

8. The objection to the specification for the improper use of trademarks is withdrawn in view of the amendments to the specification on pp. 2-4 and Applicant's comments on p. 10 of the Response of 9/19/07.

9. The objection to the specification for failing to provide sequence identifiers for the sequences KKKR and RRRR (p. 14, [043]) pursuant to 37 CFR 1.821 (c) and/or (d) is withdrawn in view of Applicant's prior amendment of 7/11/04 correcting the error.

Applicants' comments on p. 10 of the Response of 9/19/07 are acknowledged.

Claim Objections

10. The objection to Claim 28 for omitting to include the term "and" before the final species of the Markush group is withdrawn in view of the amended claim.

Applicants' comments on p. 10 of the Response of 9/19/07 are acknowledged.

Withdrawal of Rejections

Claim Rejections - 35 USC § 112, second paragraph

11. The rejection of Claims 1-14, 21-26 and 28-30 for the recitation "inhibiting angiogenesis or tumorigenesis in a biological sample" is withdrawn and moot for cancelled claim 14 and withdrawn for Claims 1-13, 21-26 and 28-30. Applicants' allegations on p. 11 of the Response of 9/19/07 citing the on-line Merriam Webster medical dictionary for a biological sample overcomes the rejection.

12. The rejection of Claims 1, 3-14 and 21-30 for omitting a method step for the biological endpoint or readout in determining the inhibition of angiogenesis or tumorigenesis is withdrawn and moot for cancelled Claim 14 and withdrawn for Claims 1, 3-12 and 21-30. The amendment of Claim 1 to delete inhibiting tumorigenesis renders this aspect of the rejection moot. Applicants allegations on p. 11 of the Response of 9/19/07 that numerous methods are available for assaying angiogenesis, specifically Examples 3-5 of the specification, renders the rejection moot.

13. The rejection of Claims 1-14, 21-26 and 28-30 in lacking antecedent basis for the limitation "the sample" in element b) of Claim 1, is withdrawn and moot for cancelled Claim 14. Applicants' amendment to correct the antecedency in Claim 1 overcomes the rejection.

Applicants' comments on p. 11 of the Response of 9/19/07 are acknowledged.

14. The rejection of Claim 4 for the recitation "APJ" is withdrawn in view of Applicants' allegations on pp. 11-12 of the Response of 9/19/07 that "APJ" is defined in the specification by *SEQ ID NO:* overcomes the rejection.

15. The rejection of Claim 14 for the recitation "and that interacts with APJ" is withdrawn and moot for the cancelled claim.

16 The rejection of Claims 24-30 for the recitation "wherein the biological sample is in a patient" is withdrawn and moot for cancelled Claim 27 and withdrawn for Claims 24-26 and 28-30. Applicants' allegations on p. 12 of the Response of 9/19/07 are acknowledged.

17. The rejection of Claims 6 and 30 because the Markush group recites the species "VEGFs, FGFs" is withdrawn in view of the amendment of the claims to insert species of VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-D, PIGF, FGF-1 and FGF-2.

Applicants' allegations on p. 12 of the Response of 9/19/07 are acknowledged. However, Applicants' amendment to insert the species VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-D, PIGF, and FGF-1 raises new grounds for rejection discussed below.

Claim Rejections - 35 USC § 112, first paragraph

Written Description

18. The rejection of Claims 8 and 14 under 35 U.S.C. 112, first paragraph, in lacking written support for the anti-apelin antibodies or fragments thereof having the properties of a) inhibiting apelin activity and b) having at least 80% or at least 90% identity with the polypeptides of SEQ ID NOS: 1 (human preproapelin); 2 (human apelin-36); 3 (human apelin-17); 4 (human apelin-13) and 5 (zebrafish apelin-13) is withdrawn and moot for cancelled Claim 14. The amendment of Claim 18 to delete element f) renders the rejection moot.

19. The rejection of Claims 8 and 14 under 35 U.S.C. 112, first paragraph, in lacking written support for a polypeptide having at least 80% identity to the polypeptides of SEQ ID NOS: 1 (human preproapelin); 2 (human apelin-36); 3 (human apelin-17); 4 (human apelin-13) and 5 (zebrafish apelin-13) or a polypeptide having at least 90% identity with the polypeptides of SEQ ID NOS:1, 2, 3, 4 or 5 is withdrawn. The cancelled Claim 14 renders the rejection moot and the amendment of Claim 8 to delete element f) renders the rejection.

Enablement

20. The rejection of Claims 8 and 14 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement is withdrawn and moot for the cancelled claim 14 and withdrawn in view of the amendment of Claim 8 to delete element f).

Rejections Maintained

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Enablement

21. The rejection of Claims 1-13, 21-26, and 28-30 under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement is maintained.

In the Office Action of 4/19/07, the grounds for rejection were based on the breadth of claim scope for any apelin inhibitor having any anti-angiogenic (or anti-tumorigenic [now deleted]) effect under any conditions in any subject including a human.

A) The specification and prior art is not enabling for apelin antisense therapy

In the Office Action of 4/19/07, the Examiner acknowledged the working examples in the specification for inhibiting vascular growth or angiogenesis in a frog embryo with antisense DNA for apelin (Example 5), apelin expression being increased in approximately one third of 154 human tumor samples compared to non-tumor tissue based on dot-blot hybridization analysis with labeled cDNA probe for human apelin (Example 6) and upregulation of apelin under hypoxic conditions in primary rat

cardiomyocte cells strongly suggestive for apelin's role in tumor angiogenesis (Example 7). Applicants' specification demonstrates functional activity for one single embodiment, an apelin antisense molecule decreasing vascular permeability in a CAM assay.

Applicants allege on p. 14, ¶2 of the Response of 9/19/07 "the specification does in fact disclose a model suggesting that an apelin inhibitor could be administered to a human patient in order to inhibit angiogenesis or tumorigenesis. Example 5 shows that an apelin antisense oligonucleotide inhibits angiogenesis in the angiogenesis model system of Xenopus embryos." Further, the Declaration of Dr. Kreig (sec. 6) asserts "an apelin antisense oligonucleotide does in fact inhibit angiogenesis" in an art-accepted model.

With respect to the use of antisense molecules, at the time the instant invention was filed, the art recognized significant unpredictability to equate phenotypes derived from antisense technology with phenotypes derived from true loss-of-function methods. According to **Stein** (**Pharmacology and Therapeutics 85: 231-236, 2000**):

> "[A]ntisense oligonucleotide biotechnology has entered a phase of its development in which many problems engendered by non-sequence specificity are being recognized and being actively addressed. However, in order to improve specificity of the methodology, attention must now also be aid to co-suppression of gene activity due to irrelevant cleavage." Stein further states that "[T]o the extent that this issue also is addressed, correlations between the down-regulation of a defined target and an observed biological outcome (e.g., growth suppression) *eventually* [*emphasis added*] may be possible." (page 235, Concluding remarks)

Stein clearly suggests that use of antisense oligonucleotide therapeutics are highly unpredictable due to "irrelevant cleavage" as a result of the low stringency

requirements for RNAse H activity, wherein a 5-base complementary region of oligomer to target may be sufficient to elicit RNAse H activity (see Stein, abstract).

Stein also teaches (**J. Clinical Investigation 108(5): 641-644, 2001**) that:

> "serious question have arisen as to whether an observed biological effect in an antisense experiment has indeed been produce by an antisense mechanism, or whether it is due to a complex combination of non-sequence specific effects. Investigators must therefore understand how to employ antisense technology properly and should recognize its limitations" (page 641, column 1, paragraph 2). However, in many, and perhaps most of the citations in which only a single oligomer was evaluated, the results reported may represent some combination of true antisense effects with sequence-nonspecific and cytotoxic effects" (page 642, column 1, lines 20-25). Except under rare and strongly justified circumstances, the use of an observed biological endpoint to claim antisense efficacy is not acceptable (page 642, column 2, lines 6-10).

Stein teaches several guidelines that reflect the state of the art at the time of filing of the instant application, including: (a) that although computer-based approaches are being developed, it is still necessary to choose the optimal antisense oligonucleotide sequence from a panel of oligonucleotides, e.g. by mRNA "walking", (b) down-regulation of a relevant molecular target must be demonstrated, and (c) maximizing sequence specificity and minimize sequence non-specificity.

Stein teaches that only approximately one in eight (12.5%) of the putative antisense oligonucleotides tested can be shown to be active (page 642, column 1, lines 14-18). Other useful controls include:

> (i) the use of two or more oligonucleotides of different sequences that are complementary to the same target. If the observed phenotype(s) are the same or distinct from those seen using control oligonucleotides, an antisense mechanism of target downregulation is strengthened, (ii) introduction of the target gene with one or more mutations in the region complementary to the antisense oligonucleotide. Lack of antisense inhibition in this case is suggestive, particularly

if the antisense oligomer is still effective when the wildtype target is forcibly over-expressed (page 642, column 1, lines 40-65).

Caplen (Gene Therapy 11(16): 1241-1248, 2004) addresses the degree of unpredictability in the art when choosing a biologically effective antisense sequence, stating that "it is unclear at this time (2004) what the minimum level of homology required between the siRNA and the target to decrease gene expression is, but it has been reported that matches of as few as 11 consecutive nucleotides can affect the RNA levels of a non-targeted transcript" (page 1245, column 2). This is especially relevant in mammalian cells because mammalian cells have nonspecific dsRNA-triggered responses primarily mediated through interferon-associated pathways that are absent in invertebrates and plants. While RNAi appears to be easy to induce, critical analysis of RNAi derived phenotypic data should not be overlooked. The validation of the RNAi effect in mammalian cells is important and that non-specific effects of RNAi need to be carefully assessed in mammalian cells (page 1245). For example, "ensuring the specificity and quantifying the efficacy of the particular siRNA or shRNA against a clinically relevant target transcript is essential in justifying its further development."

With regard to the ability of an artisan to correlate an observed antisense RNA phenotype to a predicted phenotype using targeting vectors that knock-out, gene disruption by selective ablation is the most definitive approach. Caplen teaches that the RNAi machinery can be saturated, so there will probably be a limit to the number of different genes that can be targeted in a cell at one time (page 1244, column 1). Furthermore, Caplen expresses the importance in recognizing that there is variation in

the degree of inhibition mediated by different small interfering RNA sequences which may result in the production of different phenotypes. Thus, the disclosure of a phenotype in response to the expression of a single, structurally undefined antisense molecule (page 24, Example 4, Table 2, discussed below) cannot reasonably predict the phenotype obtained when the individual gene is totally disrupted.

Delivery

In regards to the delivery of oligonucleotide pharmaceutical compositions *in vivo*, the state of the art indicates that delivery of these oligonucleotide compositions for therapeutic purposes "remains an important and inordinately difficult challenge (**Chirila et al, Biomaterials 23:321-342, 2002**, see abstract)." At the time of filing of the instant application there were no general guidelines for successful *in vivo* delivery of antisense compounds known in the art. Problems related to the pharmaceutical use of nucleic acids in general, and antisense and siRNA nucleic acids in particular, are evident from the pre- and post-filing art. One problem is the inability to routinely deliver an effective concentration of a specific nucleic acid into a target cell, such that a target gene or miRNA is inhibited to a degree necessary to produce a therapeutic effect--in this case inhibition of RNA silencing of a gene.

Gerwirtz et al. (Blood 92(3): 712-736, 1998) for example, teach that "...delivery of oligonucleotides remains an important problem..." (page 728). "The ability to deliver ODN into cells and have them reach their target in a bioavailable form must be further investigated. Without this ability, it is clear that even an appropriately targeted sequence is not likely to be efficient." (page 728)

Jen et al. (Stem Cells 18: 307-319, 2000) provide a review of the challenges that remain before antisense-based therapy becomes routine in therapeutic settings. According to Jen et al. many advances have been made in the antisense art, but also indicate that more progress needs to be made. "One of the major limitations for the therapeutic use of AS-ODNS [anti-sense oligonucleotides] and ribozymes is the problem of delivery....presently, some success has been achieved in tissue culture, but efficient delivery for *in vivo* animal studies remains questionable". Jen et al. outlines many of the factors limiting the application of antisense for therapeutic purposes and concludes "[g]iven the state of the art, it is perhaps not surprising that effective and efficient clinical translation of the antisense strategy has remained elusive." (page 313, second column, second paragraph) It is also concluded that "[a] large number of diverse and talented groups are working on this problem, and we can all hope that their efforts will help lead to establishment of this promising form of therapy." (See page 315, last two paragraphs).

Chirila et al. (Biomaterials 23:321-342, 2002, page 327, last paragraph) teach that "[T]he *in vivo* delivery techniques chiefly used at the present, i.e. infusion or injection of naked molecules and liposomal systems, do not assure adequately long-term maintenance of ODNS [oligonucleotides] in tissues," which is required to achieve therapeutic effects. As a conclusion to the review of Chirila et al, the state of oligonucleotide based drug therapy is summarized by the statement: "the antisense strategy only awaits a suitable delivery system in order to live up to its promise."

Opalinska et al. (Nature Reviews 1:503-514, 2002) teach that: "[I]t is widely appreciated that the ability of nucleic-acid molecules to modify gene expression *in vivo* is quite variable, and therefore wanting in terms of reliability. Several issues have been implicated as a root cause of this problem, including molecule delivery to targeted cells and specific compartments within cells and identification of sequence that is accessible to hybridization in the genomic DNA or RNA". "Another problem in this field is the limited ability to deliver nucleic acids into cells and have them reach their target. Without this ability, it is clear that even an appropriately targeted sequence is not likely to be efficient. As a general rule, oligonucleotides are taken up primarily through a combination of adsorptive and fluid-phase endocytosis. After internalization, confocal and electron microscopy studies have indicated that the bulk of the oligonucleotides enter the endosome-lysosome compartment, in which most of the material becomes either trapped or degraded." (page 511, columns 1-2)

Scherer et al (Nature Biotechnology 21(12), pages 1457-1465, 2003) teach that antisense oligonucleotides (ODNs), ribozymes, DNAzymes and RNA interference (RNAi) each face remarkably similar problems for effective application: efficient delivery, enhanced stability, minimization of off-target effects and identification of sensitive sites in the target RNAs. Scherer et al teach that these challenges have been in existence from the first attempts to use antisense research tools, and need to be met before any antisense molecule can become widely accepted as a therapeutic agent.

Kurreck et al (Current Opinion Drug Discovery and Development 7(2): 179-187, 2004) teaches that "many potential sites are inaccessible for complementary

oligonucleotides due to the secondary and tertiary structures of the long RNA molecule. Furthermore, RNA-binding proteins shield some regions of the mRNA" (page 179).

Lu et al (RNA Interference Technology, Cambridge, Appasani, ed., 2005, page 303) state that "Unlike *in vitro* transfection of siRNA into cells, *in vivo* delivery of siRNA into targeted tissue in animal models is much more complicated, involving physical, chemical and biological approaches, and in some cases their combination." Therapeutic applications, however, clearly depend upon optimized local and systemic delivery of siRNA *in vivo*. "....limited reports of *in vivo* studies have indicated a lack of effective delivery methods for siRNA agents." "...the two most critical hurdles are maintaining its [siRNA] stability *in vivo* and delivery to disease tissues and cells." (page 314) Lu et al. admit that while hydrodynamic delivery of siRNA duplexes into mouse liver has proven to be quite efficient, this technique is not clinically feasible in human studies.

Samarsky et al (RNA Interference Technology, Cambridge, Appasani, ed., 2005, pages 389-394) appear to agree with Lu et al., stating that "Delivery of RNAi to target cells and tissues in mammalian organism[s] is considerably more difficult than in cultured cells. This step is likely to be a critical bottleneck in the *in vivo* application of RNAi." "One major remaining obstacle is the efficient delivery of RNAi triggers to target tissues *in vivo*." (page 394)

Sioud (RNA Silencing, Methods and Protocols, Humana Press, 2005) expresses similar reservations, specifically with respect to the use of cationic carriers, as currently claimed in claims 34 and 35. On page 238, Sioud states "Despite some

encouraging results, however, liposomes still have not the characteristics to be perfect carriers because of toxicity, short circulation time, and limited intracellular delivery for target cells." And on page 243, "The *in vivo* uptake of siRNAs can differ dramatically with cell types as well as with the status of cell differentiation." "...certain synthetic siRNAs activated the production of TNF-alpha and interleukin (IL)-6 in human freshly isolated monocytes...

Similarly, **Simeoni et al (RNA Silencing, Methods and Protocols, Humana Press, 2005, page 251**) state "So far, although siRNA transfection can be achieved with classical laboratory-cultured cell lines using lipid-based formulations, siRNA delivery remains a major challenge for many cell lines and there is still no reasonably efficient method for *in vivo* application."

Mahato et al. (Expert Opinion on Drug Delivery, January 2005, Vol. 2, No.1, pages 3-28) teach that antisense oligodeoxynucleotides and double-stranded small interfering RNAs have great potential for the treatment of many severe and debilitating diseases. Mahato et al. teach that efforts have made significant progress in turning these nucleic acid drugs into therapeutics, and there is already one FDA-approved antisense drug in the clinic. Mahato et al. teach that despite the success of one product and several other ongoing clinical trials, challenges still exist in their stability, cellular uptake, disposition, site-specific delivery and therapeutic efficacy. Mahato et al. teach that in order for siRNAs to be used as therapeutic molecules several problems have to be overcome, including: the selection of the best sequence-specific siRNA for the gene to be targeted and the ability to minimize degradation in the body fluids and tissues.

The efficacy of antisense-based therapies hinges upon the ability to deliver a sufficient amount of oligonucleotide, to the appropriate tissues, and for a sufficient period of time, to produce the desired therapeutic effect. So far, it appears that all of the developments in antisense-based therapies have not been sufficient to overcome this one basic obstacle, drug delivery. The art teaches that the behavior of oligonucleotide-based compositions and their delivery *in vivo* are unpredictable, therefore claims to pharmaceutical compositions and methods of treating diseases by the administration of oligonucleotide-based pharmaceuticals are subject to the question of enablement due to the high level of unpredictability associated with this technique as taught in the prior art.

In view of the express teachings of the art suggesting that *in vivo* delivery of siRNA is unpredictable, it is essential that the instant application provide enabling disclosure showing how to use the pharmaceutical compositions of the instant invention to target any desired gene in any cell in any animal to effect the desired outcome. The skilled artisan would not know *a priori* whether introduction of oligonucleotides *in vivo* by the broadly disclosed methodologies of the instant invention, would result in the oligonucleotide reaching the proper cell in any tissue in any organism such as any mammal, including humans, in a sufficient concentration and remaining for a sufficient time to activate target-specific RNA interference of any desired gene. Specific guidance would be required to teach one of skill in the art how to deliver single-stranded small interfering RNA molecules to cells *in vivo* to produce a measurable effect in an organism. Due to differences in the physiological conditions of a cell *in vitro* versus *in vivo*, the uptake and biological activity observed *in vitro* would not predictably translate

to *in vivo* results. Cell culture examples are generally not predictive of *in vivo* inhibition, and the methods of delivery to a cultured cell, e.g., *D. melanogaster* embryos and worms, is not expected to be routinely applicable to the delivery of oligonucleotides to all other organisms, including mammals. The state of the art is such that successful delivery of oligonucleotide sequences *in vivo* or *in vitro*, such that the oligonucleotide provides the requisite biological effect to the target cells/tissues/organs, must be determined empirically.

The Existence of Working Examples and The Amount of Direction Provided by the Inventor

At the time of filing of the instant application, no general guidelines for successful *in vivo* delivery of antisense compounds known in the art, nor are such guidelines provided in the specification as filed. The specification provides general, not specific, guidelines regarding i) an amount of single-stranded small interfering RNA "sufficient for degradation of the target mRNA to occur, thereby activating target-specific RNAi in the organism", ii) physical methods of introducing nucleic acids into an organism, and iii) pharmaceutical compositions formulated to be compatible with its intended route of administration.

A review of the instant application fails to find adequate representations or guidance exemplifying the *in vivo* applications currently contemplated for which the pharmaceutical compositions are intended. There are no working examples wherein Applicants have successfully delivered the inventive ss-iRNA nucleic acid molecule(s) to an animal *in vivo*, wherein an angiogensis-associated diseased state was successfully

treated, and wherein the treatment effects were directly correlated with the administration of the inventive ss-RNA nucleic acid molecule(s) to said animal *in vivo*. Instead, the single working example is directed to a method for reducing angiogenesis in a CAM assay.

No technical guidance or exemplary disclosure is provided regarding the use of the claimed methods for targeting genes in living organisms, including any mammal, which is the subject of the invention. As the art indicates, *in culture* results are not readily extrapolated to *in vivo* applications. Furthermore, Applicant contemplates that the method of treating a disease or disorder may include the application or administration of a therapeutic agent to an isolated biological sample from a patient. However, the specification fails to disclose how the administration of a single-stranded RNA molecule *ex vivo* to a sample derived from a patient suffering from a disease will effect the treatment of a disease *in the organism* [emphasis added], e.g., a disease process that is cell-autonomous, such as an inborn error of metabolism, that will ameliorate the symptoms of the organism suffering from said cell-autonomous disease.

The specification does not provide the guidance required to overcome the art-recognized unpredictability of using nucleic acids in therapeutic applications. The teachings of the prior art does not provide that guidance, such that the skilled artisan would be able to use the claimed methods in the manner disclosed to produce the intended effects of activating target-specific RNA interference to treat angiogenesis or a disease or disorder associated with the activity of apelin protein. Furthermore, Applicant's specification does not provide actual working examples or guidance so that

the skilled artisan can deliver the pharmaceutical compositions of the claimed invention to target tissues successfully, to produce the desired therapeutic result without undue experimentation.

The Quantity of Any Necessary Experimentation to Make or Use the Invention

Therefore, the specification does not describe the use of single-stranded siRNA molecules for the *in vivo* treatment of a disease or condition associated with the expression of a target protein, in a sufficient manner so as to enable one of ordinary skill in the art to practice the present inventive methods without undue experimentation. The quantity of experimentation required to practice the invention as claimed would require determining modes of delivery in a whole organism such that a single gene is inhibited and the desired secondary effect (treatment leading to the amelioration of conditions associated with the expression of a target protein in a patient) is obtained. The specification as filed provides no specific guidelines in this regard. The deficiencies in the specification would constitute undue experimentation since these steps must be achieved without instructions from the specification before one is enabled to practice the claimed invention. For example, the instant application does not appear to teach one of skill in the art how to effectively target tissues and cells in any mammalian sample in vitro or in vivo. Similarly, while the instant application is enabling for the use of single-stranded siRNA *in ova* (e.g., birds), it does not enable the use of these RNAs *in vivo* in other multicellular organisms, such as mammals, including humans.

Thus, considering the breadth of the claims, the state of the art at the time of filing, the level of unpredictability in the art, and the limited guidance and working

examples provided by the instant application, the Examiner submits that the skilled artisan would be required to conduct undue, trial and error experimentation to practice the claimed invention(s) commensurate with the claimed scope. In conclusion, the specification fails to provide any guidance as to how an artisan would have dealt with the art-recognized limitations of the claimed method(s) commensurate with the scope of the claimed invention(s). Accordingly, the instant claims for apelin antisense molecules are rejected for failing to comply with the enablement requirement.

B) The specification and prior art is not enabling for inhibiting angiogenesis with any apelin antibody in any subject much less a human

In the Office Action of 4/19/07, the Examiner acknowledged the specification teaching "Other apelin antagonists are antibodies and fragments thereof" at [0027]. The specification does not demonstrate any working models for an inhibitory apelin antibody more especially one that interferes with apelin peptide/receptor interaction or apelin peptide/APJ interaction. The specification does not provide enablement for inhibiting angiogenesis with any antibody recognizing apelin peptides of SEQ ID NOs:1-5, or treating a biological sample in a human having any angiogenesis- associated disorder with any apelin inhibitor much less any one apelin antibody recognizing peptides of SEQ ID NOs:1-5.

Applicants' allege that "antibodies were known at the time of filing that specifically bind apelin (citing Kleinz et al., Regul. Peptides 118:119-125 (2004))" (p. 15 of the

Response of 9/19/07). The Declaration of Kreig (sec. 9) alleges that "others have identified additional antibodies that specifically bind apelin."

Applicants have not provided a copy of the Kleinz reference to verify the assertion that the antibodies of Kleinz could inhibit apelin activity, block apelin peptide receptor interactions or block apelin interaction with APJ much less whether any of the Kleinz antibodies were shown to inhibit angiogenesis in a relevant mammalian model.

Applicants' response is incomplete.

Applicants' allege that the Declaration of Kreig demonstrates an apelin antibody that specifically inhibits angiogenesis in the CAM assay (p. 15 of the Response of 9/19/07). The Declaration of Kreig alleges that four anti-apelin antibodies were produced (ab206, ab207, ab208 and ab210), that two antibodies significantly reduced endothelial cell proliferation in vitro (ab208 and ab210; Exhibit A) and the ab208 Ab inhibited angiogenesis in the CAM assay (Exhibit B).

The Examiner respectfully submits that Applicants have not disclosed which of the apelin epitopes the antibodies were made to, thus one of skill could not reasonably conclude that the ab206, ab207, ab208 and ab210 could bind to any one or more of peptides of SEQ ID NO: 1-5. Further, the specification and declaration evidence does not demonstrate that apelin antibodies that do bind peptides of SEQ ID NOs:1-5 can generate anti-angiogenesis responses in any sample from any subject, in any species and to what degree. Applicants have not shown whether any apelin antibodies were effective at blocking angiogenesis in a relevant mammalian model, again instead relying on *in ova* data from birds. Applicants have not shown biodistribution data, or with dose

response data what if any effective levels of antibody therapy could be achieved in vivo for any given disorder associated with angiogenesis in a mammalian in vivo model.

Applicants have not addressed the cited references of record as they apply to administering any antibody immunotherapy in vivo.

C) The specification and prior art is not enabling for inhibiting apelin activity in a human with an antibody against zebrafish apelin (SEQ ID NO:5)

In the Office Action of 4/19/07, the Examiner questioned the relevancy of an anti apelin antibody binding to a peptide of SEQ ID NO:5 from zebrafish apelin, more especially in practicing the instant claimed methods of treating angiogenesis in a human.

Applicants did not address this aspect of the rejection. The Declaration of Dr. Kreig does not address this aspect of the rejection.

Applicants' response is incomplete.

New Grounds for Rejection

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

22. Claims 6 and 30 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claims 6 and 30 have been amended to recite species of "VGFs" and "FGFs" that were otherwise rejected in the previous office action for indefiniteness of the terms. Now each of the claims recites "VEGF (VEGF-A), VEGF-B, VEGF-C, VEGF-D, VEGF-E, PIGF, acidic fibroblast growth factor (FGF-1)" for the species of angiogenic factor and none of which find literal support in the specification.

The specification does not literally contemplate any of these species, only that the angiogenic factor comprises VEGFs or FGFs. It appears that Applicants are asserting that official notice be taken regarding the species in the absence of supporting documentation that any one of the claimed species is in fact an angiogenesis factor. Applicants are required to identify literal support for the claimed species in the instant specification or priority applications and/or to supplement the record with literature

references substantiating the art recognized angiogenic activity for the claimed species as known "VEGFs" or "FGFs".

Conclusion

23. No claims are allowed.

24. **THIS ACTION IS MADE FINAL.** Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

25. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Lynn Bristol whose telephone number is 571-272-6883. The examiner can normally be reached on 8:00-4:00, Monday through Friday.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Larry Helms can be reached on 571-272-0832. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

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